refer to the aggregate contents of the subsamples or chambers after partition, as well as before partition.

[0060] When a sample containing a complex mixture of nucleic acid molecules it is partitioned into very small-volume sub-samples, the effective concentration of the target sequence in the sub-sample(s) in which it is located is significantly increased. Effective concentration of the target occurs because, while the number of molecules of target in the sample does not change as a result of the partitioning, the number of other molecules (including molecules that can produce side reactions, e.g., primer-dimers and non-complementary DNA sequences in the sample) is linearly proportional to volume. For example, if a 30 microliter sample containing one molecule of interest is partitioned into ten thousand subsamples (each with a volume of 3 nanoliters) the effective concentration of molecule of interest is enriched by a factor of 10^4 in the chamber in which it is located. Since the ratio of target to side reactions is inversely proportional to volume, partitioning into a small volume increases this ratio (i.e., effectively concentrates). As noted by McBride et al., such an increase in effective concentration results in remarkable sensitivity and fidelity of PCR-based detection.

[0061] Typically the sample is partitioned into at least about 10^3 different subsamples or reaction chambers, sometimes at least about 5×10^3 different sub-samples or chambers, sometimes 10^4 different sub-samples or chambers, often at least about 2×10^4 *different sub-samples or chambers, sometimes at least about* 3×10^4 different sub-samples or chambers, and sometimes at least about 10^5 different sub-samples or chambers. In certain embodiments the sample is partitioned into between 100 and 100,000 sub-samples, more often between 1000 and 50,000 sub-samples, and sometimes between 1000 and 20,000 sub-samples.

[0062] Typically the volume of each sub-sample is less than about 1000 picoliters (pL), often less than about 500 pL, sometimes less than about 100 pL, and sometimes less than about 50 pL.

[0063] The relationship between the number of nucleic acid molecules (or non-nucleic acid macromolecules, particles or cells) in a sample, the number of chambers into which the sample is partitioned, and the distribution of number of nucleic acid molecules or other entities in each chamber can be estimated using well know methods. For example, to determine the number of chambers (C) into which the number (N) particles (e.g. cells, nucleic acid molecules, etc.) would be partitioned so that most or essentially all of the chambers contained either 0 or 1 particle can be determined using a Poisson Distribution:

$$P(x,\lambda)=\frac{e^{-\lambda}\lambda^{x}}{x!}. \qquad [1]$$

$P(x,\lambda)$ is the probability of finding x particles if the average number of particles in a box is λ. We can get this by setting the average number (λ) such that

$$P(0,\lambda)+P(1,\lambda)\geq x$$

$$\frac{e^{-\lambda}\lambda^{0}}{0!}+\frac{e^{-\lambda}\lambda^{1}}{1!}\geq x$$

-continued

$$e^{-\lambda}+e^{-\lambda}\lambda\geq x$$

$$e^{-\lambda}(1+\lambda)\geq x$$

The average number of particles in a box is λ=N/C, so if you know λ and N, you can find C:

$$C=\frac{N}{\lambda}$$

λ is easily determined using eqn. [1] above.

For instance, if x is 0.99 (i.e. 99% chance of a chamber containing a 0 or 1 particle),

$$e^{-\lambda}(1-\lambda)\geqq 0.99$$

$$\lambda\leqq\sim 0.1487$$

If N is 10,000, then

$$C\cong\frac{10000}{0.1487}$$

[0064] C 67250 chambers are required to have 99% likelihood of 0 or 1 particles per chamber. Although this calculation is provided for illustration it will be understood that any method (emperical or analytical) may be used. In some applications it will be useful to use such a calculation and adjust (e.g., dilute) the sample and/or select a MPD with an appropriate number of chambers for an increased likelihood there will be few, if any, chambers with more than a predetermined number of target molecules (e.g., 1) per chamber.

[0065] iii) Amplification of Partitioned Nucleic Acids

[0066] Following the partitioning step, any target sequences of interest that are in the sample are amplified. As used herein, nucleic acid "amplification" is a process that produces multiple nucleic acid molecules (called "amplicons") based on the presence of a particular target sequence. Most often the amplicons include a base sequence that is the same as, or complementary to, the target sequence so that amplification means that the number of copies of the target sequence increases. These identical or complementary amplicons are the products, for example and without limitation, of the Polymerase Chain Reaction (PCR) [see, Dieffenbach and Dvksler, 1995, PCR Primer: A Laboratory Manual. CSHL press, Cold Spring Harbor, USA]; Nucleic Acid Sequence Based Amplification (NASBA) [see Sooknanan and Malek, 1995, *BioTechnology* 13:563-65] SPIA™ Isothermal Linear Amplification, Ribo-SPIA, X-SPIA™ [Nugen Technologies, San Carlos Calif., see U.S. Pat. No. 6,251,639, WO 02/72772; US2003/0017591 A1]; the Ligase Chain Reaction (LCR) [Wu and Wallace, 1989, Genomics 4:560; Landegren et al., 1988, *Science* 241:1077]; Transcription amplification [Kwoh et al., 1989, *Proc. Natl. Acad. Sci. USA* 86:1173]; Self-sustained sequence replication [Guatelli et al., 1990, *Proc. Nat. Acad. Sci. USA* 87:1874]. In some embodiments, however, an amplicon is a nucleic acid with a sequence different from the target sequence and the process of amplification consists of increasing the number of amplicons if the target is present in a chamber, but not in the absence of the target sequence.